Synthetic lethality – the road to novel therapies for breast cancer

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Abstract

When the *BRCA1* and *BRCA2* tumour suppressor genes were identified in the early 1990s, the immediate implications of mapping, cloning and delineating the sequence of these genes were that individuals in families with a *BRCA* gene mutation could be tested for the presence of a mutation and their risk of developing cancer predicted. Over time though, the discovery of *BRCA1* and *BRCA2* has had a much greater impact than many might have imagined. In this review, we discuss how the discovery or *BRCA1* and *BRCA2* has informed not only an understanding of the molecular processes that drive tumourigenesis, but has also reignited an interest in therapeutically exploiting loss of function alterations in tumour suppressor genes.

1. BRCA1 and BRCA2 – discovery, genetics and biology

Globally, breast cancer is the most common cancer in women. Its familial form constitutes 5-10% of all breast cancers and has a dominant mode of inheritance and is characterised by earlier onset of disease, relative to breast cancer in the general population. Heterozygous germ-line mutations in either the *BRCA1* or *BRCA2* tumour suppressor genes are the most common genetic cause of familial breast cancer, and were identified as breast cancer susceptibility genes in the 1990s through linkage analysis in families with the disease (Futreal, et al. 1994), (King 2014), (Miki, et al. 1994), (Wooster, et al. 1995), (Tavtigian, et al. 1996). *BRCA1* and *BRCA2* mutations are found in 25% -28% of familial breast cancers and mutation carriers have a lifetime risk of 40-87% for developing breast cancer by the age of 70. Mutation carriers also have a lifetime risk of 45-60% (*BRCA1* mutation carriers) or 11-35% (*BRCA2* mutation carriers) for developing ovarian cancer (Ford, et al. 1998; King, et al. 2003). Other types of cancers also found in *BRCA1* and *BRCA2* mutation carriers include pancreatic and prostate cancers (Antoniou, et al. 2003; Breast Cancer Linkage 1999; Edwards, et al. 2003; King et al. 2003; Ozcelik, et al. 1997; van Asperen, et al. 2005). Although most familial *BRCA1* or *BRCA2* mutations are inherited as heterozygous mutations, rare, biallelic germ-line mutations do occur in patients with Fanconi anemia (Domchek, et al. 2013; Howlett, et al. 2002; Meyer, et al. 2014; Sawyer, et al. 2015). Furthermore, in addition to germ-line mutations in these genes, somatic *BRCA1* and *BRCA2* mutations are also found in breast, prostate, ovarian and pancreatic cancers, as is somatic hypermethylation of the *BRCA1* gene promoter. An analysis of tumours from individuals with *BRCA1* or *BRCA2* mutations indicates that the wildtype allele is generally lost (Collins, et al. 1995; Futreal et al. 1994; Gudmundsson, et al. 1995), suggesting that loss of heterozygosity at the *BRCA1* and *BRCA2* loci appears to be an important event for tumourigenesis.

Both *BRCA1* and *BRCA2* are very large genes, which display limited sequence homology to each other. *BRCA1* is comprised of 24 exons that translate to a 1863 amino acid protein with a RING domain with E3 ubiquitin ligase activity, a coiled-coil domain in the largely unstructured central region important for binding with another tumour suppressor protein, PALB2, and BRCT (BRCA1 carboxy terminal) repeats important for interaction with phosphorylated proteins (Brzovic, et al. 2001; Sy, et al. 2009; Wu, et al. 1996; Xia, et al. 2006; Zhang, et al. 2009b). *BRCA2* is comprised of 27 exons that translate to a 3418 amino acid protein that includes amino-terminal BRC repeats, which mediate binding of BRCA2 to PALB2 and the DNA recombinase RAD51, a central DNA binding domain, and nuclear localization and RAD51 control domains at the carboxy-terminus (Sharan, et al. 1997; Wong, et al. 1997; Yang, et al. 2002).

Small insertions/deletions (in-dels) or nonsense mutations leading to truncations are the most common *BRCA* gene mutation type observed in cancer patients. There are at least 1790 distinct mutations, polymorphisms and variants that have been identified in *BRCA1* to date and over 2000 in *BRCA2* according to the Breast Cancer information Core (BIC) and ClinGen. Mutations are distributed across the entire coding sequence for both genes with over 50% of observed mutations being private to particular individuals. In addition to known pathogenic mutations, there are a large number of missense, in-frame deletion and silent mutations known as “variants of unknown significance” which have unclear pathogenic potential, making clinical interpretation of genetic testing difficult in cancer patients harboring these.

Breast tumours in *BRCA1* mutation carriers tend to have a basal-like transcriptional signature and more often than not exhibit a “triple negative” phenotype, lacking expression of the estrogen and progesterone receptors and lacking amplification of

the *ERBB2* (*HER2*) oncogene (Foulkes, et al. 2003). This triple negative phenotype precludes the use of targeted estrogen receptor-based or ERBB2-specific therapies and in general, BRCA1 mutant breast cancers are treated with traditional genotoxic chemotherapy agents. In contrast, breast tumours in *BRCA2* mutation carriers tend to better reflect the hormone receptor and ERBB2 status of breast cancers in the non-BRCA mutant population (Jonsson, et al. 2010; Waddell, et al. 2010).

2. BRCA1 and BRCA2 functions in DNA repair and replication fork protection

Tumourigenesis occurs in the absence of BRCA1 and BRCA2 as both proteins play important roles in the repair of DNA double-strand breaks (DSBs) (Moynahan and Jasin 2010) and the stability of replication forks. DSBs, the most toxic type of DNA lesions, can be catastrophic for the cell if left unrepaired as they compromise the double helix structure of DNA. The two main methods of DSB repair are the error-prone non-homologous end-joining (NHEJ) pathway and the error-free homologous recombination (HR) pathway. NHEJ, used predominantly in the G_1 phase of the cell cycle, can result in loss of genetic information proximal to the DSB site. In contrast, HR, active during S and G_2 phases, uses homologous sequence from a sister chromatid for error-free repair of DSBs. In HR, after the initial detection of the DSB, the broken DNA ends are enzymatically resected to generate 3' single-stranded DNA (ssDNA). The ssDNA is coated by the Replication Protein A (RPA) complex, which is then replaced by the RAD51 recombinase. The binding of multiple RAD51 molecules onto ssDNA enables strand invasion, where ssDNA from the damaged DNA site invades the double helix of intact DNA, a process that facilitates the identification of a homologous DNA sequence that is used as a template for DNA repair across the break site. Efficient resolution of the resulting intermediates completes the process with the genetic integrity of broken DNA restored.

BRCA1 and BRCA2 play key roles in HR. BRCA1 is required for CtIP-mediated resection of DSBs to generate single-stranded DNA (ssDNA) which is coated by the RPA complex (Chen, et al. 2008; Yu and Baer 2000). BRCA1-mediated resection is a key step in committing to repair by HR as opposed to the error-prone NHEJ pathway (Kass and Jasin 2010). CDK-phosphorylated CtIP protein binds BRCA1 BRCT repeats, is localised to the DSB and mediates resection through the MRN (MRE11-RAD50-NBS1) complex (Chen et al. 2008; Sartori, et al. 2007; Wong, et al. 1998; Yu, et al. 1998). In addition to CtIP localization to DSBs, BRCA1 also counteracts 53BP1 function, and in doing so impairs NHEJ (Bouwman, et al. 2010; Bunting, et al. 2010). Additionally, both BRCA1 and BRCA2, bridged by PALB2, are required for the recruitment of the DNA recombinase RAD51 to damaged DNA, where it forms a nucleoprotein complex (or "filament") with ssDNA that mediates strand invasion (Sy et al. 2009; Tischkowitz and Xia 2010; Xia et al. 2006; Zhang, et al. 2009a; Zhang et al. 2009b). BRCA2 is not only required for localization of RAD51 to RPA-coated DNA but also for stabilizing the RAD51 nucleofilament that is formed by blocking RAD51-mediated ATP hydrolysis.

When the progression of replication forks is halted (replication fork stalling), which can be caused by a variety of factors including damaged DNA lesions being encountered by the replication fork or the relative absence of the requisite nucleotides, preventing the disintegration or collapse of the fork structure is key to the continued fitness of cells. One of the molecular events that challenges fork stability in this setting is the activity of the nuclease MRE11, which if not tightly controlled, degrades newly synthesised (nacent) DNA at the replication fork, potentially forcing fork collapse. In addition to their roles in DSB repair, BRCA1 and BRCA2 prevent the degradation of nascent DNA at stalled replication forks (Pathania, et al. 2014; Schlacher, et al. 2011; Schlacher, et al. 2012). For example,

Schlacher and colleagues found using single-molecule DNA fibre analysis that once replication forks are stalled with hydroxyurea (HU), tracts of nascent DNA produced prior to fork stalling are degraded in the absence of BRCA2 by MRE11. This protection of nascent DNA at replication forks appears to be mediated by a conserved C-terminal region in BRCA2 that stabilises RAD51 nucleoprotein filaments but is not required for RAD51 loading or homologous recombination *per se* (Schlacher et al. 2011). Using Brca1-deficient embryonic stem (ES) cells, Schlacher and colleagues later found that Brca1 also prevents fork degradation by MRE11 (Schlacher et al. 2012).

The loss of DNA repair and fork stability functions of BRCA1 and BRCA2 are likely causes of the genomic instability seen in *BRCA1* or *BRCA2* mutant tumours. Cells deficient in either protein have been shown to have reduced efficiency of HR (Moynahan, et al. 2001a; Moynahan, et al. 2001b). BRCA1/2-deficient cells also exhibit spontaneous and DNA-damage induced genetic instability, which subsequently contributes to tumourigenesis. Additionally, BRCA1/2-deficient cells are sensitive to DNA damaging agents, especially those that form crosslinks on DNA such as cisplatin (Narod 2010). This particular phenotype has been exploited in the clinic to treat BRCA-deficient tumours. In fact, cisplatin and its derivative, carboplatin, have been shown to be particularly effective in treatment of *BRCA1* and *BRCA2*-associated cancers, particularly in ovarian cancers (Boyd, et al. 2000; Cass, et al. 2003; Chetrit, et al. 2008; Tan, et al. 2008; Vencken, et al. 2011). However, chemoresistance to platinum compounds is a very significant clinical problem and has a negative impact on patient survival. Therefore, identification of additional drugs that can effectively treat HR-deficient cancers is essential.

3. Therapeutic exploitation of *BRCA* gene defects with PARP inhibitors

The intricate dissection of *BRCA1* and *BRCA2* function, and in particular the discovery that these tumour suppressor proteins were required for effective HR, made a significant impact upon the discovery and mechanistic understanding of therapeutic approaches that target *BRCA1* or *BRCA2* (*BRCA1/2)* gene mutant cancers. To date, the majority of agents proposed to selectively inhibit *BRCA1/2* mutant tumour cells likely do so by causing the stalling and collapse of DNA replication forks. Specifically, these agents cause replication fork damage that requires HR for repair (Figure 2). In the absence of *BRCA1* or *BRCA2* gene function, and therefore functional HR, tumour cells most likely attempt to repair replication forks via non-HR forms of DNA repair; these alternative repair strategies cause large scale chromosomal abnormalities which ultimately impair the fitness of cells and induce cell death. Early evidence of this phenomenon was suggested by work illustrating the sensitivity of *BRCA* gene defective cells to platinum salts (Bartz, et al. 2006; Evers, et al. 2008; Fedier, et al. 2003) or topoisomerase inhibitors such as camptothecin (Rahden-Staron, et al. 2003). Platinum salts most likley stall replication forks by causing intra- and interstrand crosslinks in DNA through covalent interaction with nucleophilic N-7 sites on purine residues (Sikov 2015); these “lesions” within the DNA structure prevent normal unwinding of the DNA double helix prior to replication (Figure 2, (Sikov 2015)). DNA topoisomerase enzymes bind DNA and unwind its helical structure (Champoux 2001), a prerequistite for multiple processes such as DNA replication, transcription, recombination and chromatin remodeling (Champoux 2001); topoisomerase inhibitors (also known as topo-poisons) such as camptothecin, fix or “trap” topoisomase on DNA (Lord and Ashworth 2012; O'Connell, et al. 2010). Presumably, this trapped form of topoisomerase provides a bulky structure which prevents the progression of the replication fork (Figure 2, (O'Connell et al. 2010)).

In *in vitro* tissue culture models at least, platinum salts and topoisomerase inhibitors selectively target *BRCA1/2* gene mutant tumour cells, compared to cells with "wild type" function, but still have relatively profound inhibitory effects on wild type cells (Evers et al. 2008). Conversely, work from two teams in 2005, suggested that small molecule inhibitors of the DNA repair enzyme, poly (ADP-ribose) polymerase (PARP) caused profound cell inhibitory effects in *BRCA1* (Farmer, et al. 2005) or *BRCA2* mutant (Bryant, et al. 2005; Farmer et al. 2005) tumour cells but had minimal effects in wild type cells with functional HR. PARP1, is an enzyme that uses β-NAD+ as a co-factor to synthesise poly (ADP-ribose) chains (PAR) on target proteins and has a known role in the repair of single strand DNA breaks (breaks in one strand of the DNA double helix) (Hottiger, et al. 2010). At the time, it was thought that the inhibition of PARP activity might cause an accumulation of DNA damage that requires HR for its repair (Bryant et al. 2005; Farmer et al. 2005). Subsequently, this hypothesis has been refined by data suggesting that the key cytotoxic DNA lesion in PARP inhibitor exposed tumour cells is PARP "trapped" on DNA (Figure 2, (Murai, et al. 2012; Murai, et al. 2014)), a mechanism reminiscent of that used to explain the BRCA selectivity of topoisomerase inhibitors. PARP binds damaged DNA and then initiates a series of PARylation events; one of these events is autoPARylation (PARylation of PARP itself), which causes the release of PARP once its role in the initial phase of DNA repair is complete (Murai et al. 2012). It seems possible that some catalytic inhibitors of PARP impair autoPARylation, thus trapping PARP on the double helix where it is able to stall and collapse replication forks (Figure 2).

A number of years after the pre-clinical observation of PARP inhibitor/*BRCA* gene synthetic lethality, clinical trials, including those which studied breast cancer patients, confirmed the potential of PARP inhibitors as treatments for *BRCA* gene mutant cancers. Although these trials have recently been reviewed in detail elsewhere (Balmana, et al. 2011; Livraghi and Garber 2015; Lord, et al. 2015) the key trials can

be summarised as follows:

3.1 Early phase 1 trials show sustained anti-tumour responses in germ-line *BRCA* mutant cancers. Although the first-in-human PARP inhibitor clinical trial assessed the safety of rucaparib (Pfizer), olaparib (aka AZD2281, Lynparza, KuDOS/AZ) was the first PARP inhibitor to be formally assessed in *BRCA1/2* gene mutant patients. In a Phase 1 clinical trial of olaparib (Fong, et al. 2009) 60 patients were treated with 400 mg twice daily olaparib, 19 of whom had germ-line mutations in either *BRCA1* or *BRCA2*; in this subset of *BRCA1/2* gene mutant patents, 63% exhibited a clinical benefit from olaparib treatment, as defined by radiological and/or tumour marker responses or disease stabilization for a period greater than 4 months (Fong et al. 2009). Even though dose limiting myelosuppression and central nervous system side effects were seen in some patients, many of the sustained anti-tumour responses were not associated with the deleterious side effect profile normally associated with classical chemotherapy (Fong et al. 2009). On the basis of these promising results, the same phase I trial was subsequently expanded to include a total of 50 germline *BRCA1/2* mutant carriers with ovarian, primary peritoneal or fallopian tube carcinoma; here an overall response rate (ORR) of 40% and a disease control rate (DCR) of 46% was observed (Fong, et al. 2010). In a retrospective analysis of this study, a significant correlation between a good response to prior platinum salt treatment and subsequent therapeutic response to olaparib was seen (Fong et al. 2010). One explanation for this correlation is that both platinum salts and PARP inhibitors both stall replication forks and require HR for the repair of the subsequent DNA lesions caused (Figure 2).

3.2 Key phase 2 clinical trials in germ-line or somatic *BRCA* mutated cancers. The promising results from this Phase I clinical study prompted two Phase 2 clinical

trials investigating single-agent olaparib in patients with *BRCA* gene mutant chemotherapy-resistant breast (Tutt, et al. 2010) or ovarian cancers (Audeh, et al. 2010). These trials, which used either a 400 mg or 100 mg twice daily treatment regimen, established an ORR of 33% in ovarian cancer patients in the 400 mg twice daily treatment schedule and 13% of the 100 mg twice daily group, with a median progression-free survival (PFS) of 5.8 months and 1.9 months respectively (Audeh et al. 2010). Similar response rates were observed in the breast cancer cohort, where a response rate (RR) of 22% was seen in the 100 mg twice daily cohort (PFS 3.8 months), whereas a RR of 41% was observed in the cohort receiving a higher dose of olaparib (PFS 5.7 months) suggesting that the higher dose was essential in order to achieve a maximal clinical response.

As discussed later, many sporadic ovarian serous and non-familial triple negative breast cancers display many of the molecular and histopathological features found in germ-line *BRCA1/2* gene mutant tumours, which are often driven by somatic mutations in *BRCA1*, *BRCA2* and other HR-modifying genes, a concept termed 'BRCAness' (Turner, et al. 2004). On this basis, olaparib was also assessed as a monotherapy in sporadic cancers thought to display the BRCAness phenotype, namely high-grade serous ovarian cancer (HGSOvCa) and triple negative breast cancers (described earlier). In patients with HGSOvCa, both *BRCA1/2* gene mutant and non-mutant patients demonstrated a number of sustained therapeutic responses to olaparib, a number of which were also associated with prior platinum sensitivity (Gelmon, et al. 2011). In triple negative breast cancer patients, those with *BRCA1/2* gene mutations exhibited a higher frequency of disease stabilization in response to olaparib treatment than those without *BRCA1/2* gene mutations (63% versus 13%), but unlike in the ovarian cancer cohort, no sustained responses were achieved in either the *BRCA1/2*-mutant or non-mutant patients (Gelmon et al. 2011). Alongside these studies, olaparib was assessed as a maintenance therapy (i.e. a therapy used

to reduce disease recurrence after chemotherapy) in patients with HGSOvCa who had previously received carboplatin, a platinum salt chemotherapy ((Ledermann, et al. 2012) NCT00753545, Study 19). In this study, 136 patients received olaparib after chemotherapy, with 129 receiving a placebo instead. An early analysis of this trial suggested that when used as a maintenance monotherapy, olaparib significantly improved PFS, and time to first and second subsequent therapy or death compared to the use of a placebo in the maintenance setting, with *BRCA1/2* mutant patients (be it germ-line or somatic) in the trial deriving the greatest benefit from olaparib. However, an effect on OS in either *BRCA1/2* gene mutant or non- *BRCA1/2* gene mutant patients was not seen (Ledermann, et al. 2014). Nevertheless, the improvements in PFS were sufficient to warrant an approval by the FDA and EMA for olaparib as a maintenance monotherapy in HGSOv cancer characterized by *BRCA1/2* gene mutation, making this PARP inhibitor not only the first synthetic lethal treatment for cancer to be approved but also the first treatment for an inherited cancer (Kim, et al. 2015). A retrospective analysis of data from study 19, conducted after 77% of the patients had died, has now shown an overall survival benefit from olaparib maintenance monotherapy; in the *BRCA* mutant patients, this OS benefit was most pronounced (median OS 34.9 months for olaparib vs. 30.2 months for placebo, Hazard Ratio (HR) of 0.62), but was also seen in the entire dataset, which included both *BRCA1/2* mutant and non-*BRCA1/2* mutant patients (OS 29.8 months (olaparib) vs. 27.8 (placebo), HR 0.73) (Ledermann JA 2016).

Additional clinical trials observed responses to olaparib in patients with *BRCA1/2* mutations in a spectrum of other *BRCA1/BRCA2*-associated cancers including pancreatic and prostate cancers (Kaufman, et al. 2015). Notable amongst these studies has been the TO-PARP phase II clinical trial assessing the efficacy of olaparib in men with metastatic, castration-resistant prostate cancer (Mateo, et al. 2015). Out of 49 patients whom all had prior treatment (docetaxel, the androgen

synthesis inhibitor abiraterone or the androgen receptor inhibitor enzalutamide) and received oral olaparib at 400mg twice daily, 14 showed a response to olaparib; seven of these patients harboured *BRCA2* defects and four exhibited tumour specific *ATM* defects, raising the possibility that other genes involved in HR, such as ATM, might also be good predictive biomarkers of olaparib response (Mateo et al. 2015). On the basis of this study, olaparib has now been given breakthrough status in prostate cancer, and an expansion of the TO-PARP trial to a larger number of patients with HR gene defects is now underway.

Although, not all PARP inhibitor trials have delivered such positive results (Lord et al. 2015), the clinical responses in the phase 2 trials described above, alongside the favourable side-effect profile of PARP inhibitors such as olaparib, talazoparib, rucaparib, niraparib and veliparib, has provided the impetus for initiating a series of phase III trials, including those in breast cancers. It is expected that within a few years, the data from these trials will provide some of the definitive information that could support or refute the case for using PARP inhibitors in cancers other than HGSOv cancer.

4. Additional *BRCA* directed therapy

As described above, in addition to PARP inhibitors, a number of conventional chemotherapies routinely used in the management of cancer might also provide an approach to targeting *BRCA1/2* mutant tumours. These include platinum agents, topoisomerase I inhibitors (topotecan and camptothecin), topoisomerase II inhibitors (doxorubicin and etoposide) described above but also nucleoside analogous such as gemcitabine which prevents DNA synthesis when incorporated into DNA by preventing chain elongation during DNA replication (Gandhi, et al. 1996; Lord and Ashworth 2016). The common mechanism of action of these agents is that they can

stall the normal progression of replication forks and likely require *BRCA* and HR function for the repair of the DNA lesions they cause. These agents have been assessed both pre-clinically (Bartz et al. 2006; Fedier et al. 2003; Rahden-Staron et al. 2003) and clinically and have shown selectivity in *BRCA1/2* defective backgrounds (Kilburn and Group 2008; Silver, et al. 2010).

One of characteristics of *BRCA1/2* mutant tumours is an elevated mutational load, compared to non- *BRCA1/2* mutant tumours, a likely effect of defective HR. Clinical responses to immune checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 antibodies have previously been associated with hypermutated cancers, including lung carcinomas and melanomas; it seems possible that similar approaches could be used to target *BRCA* mutant tumours. To investigate this, Strickland and colleagues recently predicted neoantigen load in *BRCA1/2*-mutated HGSOv tumours and found that this was elevated compared to tumours without HR gene defects, as were the presence of CD3+ and CD8+ tumour infiltrating lymphocytes and PD-1 and PD-L1 expression in tumour-associated immune cells (Strickland, et al. 2016). Such an analysis therefore supports the clinical assessment of combinations of PARP inhibitors with PD-L1 inhibitors in breast and ovarian cancers (eg NCT02484404 - (Lee J 2016)), as does data from a pre-clinical study illustrating the efficacy of an anti-CTLA4 antibody in combination with the PARP inhibitor veliparib in a mouse model carrying a *Brca1* mutant tumour (Higuchi, et al. 2015).

5. Drug resistance in a *BRCA* mutant setting

Though PARP inhibitors have shown to be useful for the treatment of BRCA1/2-associated cancers, PARP inhibitor resistance is likely to be a major obstacle to the overall effectiveness of treatment (Fong et al. 2009; Tutt et al. 2010). PARP inhibitor resistance, in *BRCA1* or *BRCA2* mutant cancers, can occur due to reversal of

synthetic lethality by several mechanisms including restoration of DSB repair by HR, loss of PARP1 expression, loss of 53BP1 expression and upregulation of PARP inhibitor efflux from cells (**Figure 3**).

5.1 Restoration of BRCA1/2 function as a mechanism of PARP inhibitor resistance

We and others hypothesized that restoration of HR may be able to reverse chemosensitivity to DNA damaging drugs in BRCA1/2-deficient cells based on the observation that spontaneously occurring secondary genetic alterations could compensate for the initial disease causing mutations in some patients with Fanconi anemia (FA), including reversal of DNA damaging agent sensitivity in patient cells (Hirschhorn 2003; Ikeda, et al. 2003; Wiegant, et al. 2006). We hypothesized that acquired secondary intragenic *BRCA1* or *BRCA2* mutations may reverse the effect of the initial disease-causing *BRCA1/2* mutations in tumours and result in resistance to PARP inhibitors and DNA crosslinking drugs such as cisplatin and carboplatin (Edwards, et al. 2008; Sakai, et al. 2009; Sakai, et al. 2008; Swisher, et al. 2008).

Evidence for secondary *BRCA1/2* mutations as a method of reversing PARP inhibitor-related synthetic lethality was first demonstrated in several *in vitro* and *in vivo* drug-selected *BRCA2* mutated cell lines (Edwards et al. 2008; Sakai et al. 2009; Sakai et al. 2008). PARP inhibitor- or cisplatin-selected clones of the pancreatic cancer cell line CAPAN-1 (*BRCA2.6147delT*) and ovarian cancer cell line PEO1 (*BRCA2.5193C>G*) acquired secondary *BRCA2* mutations that restore the open reading frame and express functional BRCA2 protein (Edwards et al. 2008; Sakai et al. 2009; Sakai et al. 2008). PARP inhibitor-resistant clones had internal insertions or deletions in the *BRCA2* gene that eliminated the truncating effect of the parental c.6147delT mutation in CAPAN-1 cells and changed the nonsense mutation in PEO1

393 cells to a missense mutation. PEO4 ovarian cancer cells derived from the same
394 patient as PEO1 cells, after the onset of clinical resistance, were resistant to both
395 PARP inhibitor and cisplatin as a result of a secondary *BRCA2* mutation that
396 converts the parental nonsense mutation, p.Y1655X, to a silent mutation p.Y1655Y.
397 The same silent mutation was also found in the drug resistant tumour sample from
398 the same patient.
399
400 The restored BRCA2 proteins in CAPAN-1, PEO1 and PEO4 cells are functional as
401 evidenced by the restoration of ionizing radiation induced RAD51 foci formation,
402 reduced DNA damage-induced chromosomal aberrations and cross-resistance to
403 cisplatin. Non-BRCA2-restored clones of CAPAN-1 or PEO1 had neither secondary
404 BRCA2 mutations nor restoration of damage induced RAD51 foci formation.
405 Importantly, depletion of BRCA2 by siRNA reversed the drug resistance in BRCA2-
406 restored clones and ectopic expression of the mutant BRCA2 proteins found in
407 resistant clones led to drug resistance in BRCA2-deficient backgrounds.
408
409 Although the majority of pre-clinical studies identified mutations in BRCA1, and not
410 BRCA1, that were associated with therapy resistance, several clinical studies have
411 demonstrated that this is an effect that likely operates for both of the tumour
412 suppressor genes. Norquist et al evaluated PARP inhibitor response in
413 cisplatin/carboplatin-resistant ovarian tumours from patients with *BRCA1* or *BRCA2*
414 mutations (Norquist, et al. 2011). Of the three non-BRCA1/2-restored tumours, two
415 showed complete response to PARP inhibitor and one showed a partial response. As
416 expected, two of the three BRCA1/2-restored tumours with secondary *BRCA1/2*
417 mutations did not show response as the disease progressed presumably due to
418 restored HR, while the third showed partial response (Norquist et al. 2011).
419

In another study, Barber et al found evidence of secondary *BRCA2* mutations in two PARP inhibitor-resistant tumours that were not present in matched treatment naïve tumour samples from the same patients (Barber, et al. 2013). A breast tumour from a male carrying the *BRCA2c.9106C>T* nonsense mutation, had acquired a secondary mutation that changed the nonsense (p.Q2960X) to a missense (p.Q2960E) mutation. The second observation was a high grade serous ovarian carcinoma from a patient carrying the *BRCA2c.4705_4708delGAAA* mutation who was previously treated for breast cancer. In this case, the BRCA2 open reading frame was restored as a result of a larger deletion, *BRCA2c.4697_4709delAAATACTGAAAG*, which encompassed the germline *BRCA2* deletion mutation. Though not formally tested, both secondary *BRCA2* mutations likely restore at least partially functional BRCA2 protein that cancels PARP inhibitor-associated synthetic lethality (Barber et al. 2013).

In an Australian study, whole genome sequencing and analysis of high grade serous ovarian carcinomas revealed five individuals with platinum resistant disease who had secondary *BRCA1/2* mutations out of a total of ten patients analysed who had germline *BRCA1/2* mutations (Patch, et al. 2015). One of the two patients, whose tumour was also cross-resistant to PARP inhibitor, had at least 12 distinct secondary deletion mutations in *BRCA2* identified from multiple metastatic sites. The second patient had two distinct secondary *BRCA1* mutations that changed the germline nonsense mutation to missense mutations in platinum-resistant cells.

A recent study by Jonkers and colleagues has also provided strong evidence for BRCA1-restoration as an important mechanism for PARP inhibitor and cisplatin resistance in BRCA1-deficient, triple negative breast cancer (Ter Brugge, et al. 2016). The analysis of patient derived xenograft (PDX) models of triple negative breast cancer included those derived from BRCA1-deficient tumours with *BRCA1* promoter hypermethylation and a frameshift mutation leading to a premature stop

(*BRCA1.c2210delC*). In line with previous observations in *BRCA1*-mutated tumours, *BRCA1 c2210delC* therapy resistant tumours had intragenic deletions that restore the *BRCA1* open reading frame to restore BRCA1 expression and IR-induced foci formation of RAD51. Interestingly, demethylation of the *BRCA1* promoter was shown to be the major mechanism of resistance in therapy resistant tumours derived from BRCA1 promoter hypermethylated tumours. *BRCA1* gene fusions with other chromosome 17 genes also allowed the bypass of *BRCA1* promoter hypermethylation to allow BRCA1 expression in a few drug-resistant tumours. Analysis of posttreatment tumours from individuals with *BRCA1* promoter hypermethylation in pretreatment samples showed a significant decrease in BRCA1 promoter methylation which correlated with a similar increase in *BRCA1* mRNA. Taken together, data from these studies provide strong evidence of BRCA1 restoration by multiple mechanisms, including *BRCA1* promoter demethylation, as an important driver of PARP inhibitor and cisplatin resistance in BRCA1-deficient breast cancer.

Though clinical examples of secondary *BRCA1/2* mutations in PARP inhibitor-resistant breast and other cancers remain few, more extensive data are available for secondary *BRCA1/2* mutations in platinum-resistant cancers (Dhillon, et al. 2011; Norquist et al. 2011; Swisher et al. 2008). Data from cell line models and the limited clinical samples suggest that BRCA1/2-restored, platinum-resistant tumours have a high likelihood of being cross-resistant to PARP inhibitor. Additionally, secondary *BRCA1/2* mutations resulting in PARP inhibitor and cisplatin resistance are likely driven by the convergence of at least three different factors: increased mutation rate due to exposure to genotoxic agents, the lack of error-free DNA repair and a selective advantage for BRCA1/2-restored cells when patients are treated with PARP inhibitors or platinum salts.

Several examples of BRCA1/2 restoration by means other than secondary mutation also exist. For example, Johnson *et al* showed that stabilization of a normally undetectable mutant BRCA1 protein can lead to PARP inhibitor resistance in rucaparib selected clones derived from the MDA-MB-436 breast cancer cell line, harboring a *BRCA1.5396+1C>A* splice donor site mutation (Johnson, et al. 2013). Expression of an HSP90-stabilized, carboxy-terminus truncated BRCA1 protein results in restoration of damage induced RAD51 foci formation and decreased PARP inhibitor induced chromosomal aberrations. Reduced 53BP1 expression was also observed in rucaparib resistant cells which may allow increased BRCA1-independent CtIP-mediated resection, though 53BP1 loss alone was not sufficient to render cells resistant to the PARP inhibitor. The combination of mutant protein stabilization and reduced 53BP1 expression was also observed in a clinical cisplatin-resistant ovarian cancer sample though response to PARP inhibitor is unknown.

In another study, Wang *et al* demonstrated that breast cancer cell lines with mutations in the central large exon 11 of *BRCA1* express the Δ11q splice variant show partial PARP inhibitor resistance, and strong ionizing radiation-induced BRCA1 and RAD51 foci formation (Wang, et al. 2016a). Depletion of the Δ11q splice variant reduced foci formation and sensitized cells to PARP inhibitor and cisplatin. Moreover, five year overall survival in individuals with the exon 11 mutations was similar to those with wildtype *BRCA1* and worse than those carrying mutations outside of exon 11.

Several groups have provided evidence for hypomorphic activity of two additional *BRCA1* mutations that contribute to PARP inhibitor and cisplatin resistance (Drost, et al. 2011; Drost, et al. 2016; Powell 2016; Wang, et al. 2016b). The *BRCA1.C61G* mutation in the RING domain abolishes BRCA1 ubiquitin ligase activity, while still

promoting tumourigenesis. Importantly, mouse *Brca1.C61G* cells are not sensitive to PARP inhibition suggesting the mutant protein retains at least partial function. Drost et al also showed recently that a BRCA1 variant missing the RING domain ("RING-less" BRCA1) can be detected in cells from a mouse model carrying the *Brca1.185STOP* mutation and in the human breast cancer cell line SUM1315MO2 with the *BRCA1.185delAG* mutation (Drost et al. 2016). Importantly, expression of "RING-less" BRCA1 renders cells partially resistant to PARP inhibitor and cisplatin, suggesting its intact carboxy-terminus provides partial function. Moreover, Wang et al showed that PARP inhibitor and cisplatin resistant clones of the SUM1315MO2 cell line had increased expression of the "RING-less" BRCA1 variant that results from translation at an alternative start site (Wang et al. 2016b). Ectopic overexpression of this BRCA1 variant resulted in partial resistance to PARP inhibitor and cisplatin *in vitro* and *in vivo.* Interestingly, Drost et al did not observe increased expression of the "RING-less" BRCA1 consistently in cisplatin resistant clones. The existence of partial function mutants warrants a better understanding of how specific mutations impact response to PARP inhibitor, cisplatin and other therapies and the clinical management of BRCA-deficient breast and other types of cancers.

5.2 Loss of 53BP1 expression

BRCA1 and 53BP1 play important roles in choice of DSB repair by HR or NHEJ: BRCA1 promotes HR while 53BP1 tips the balance in favour of NHEJ. Several groups have shown that loss of the 53bp1 in a *Brca1-null* or *Brca1Δexon11* mice rescues embryonic lethality observed in Brca1-deficient mice (Bouwman et al. 2010; Bunting et al. 2010; Cao, et al. 2009). Brca1/53bp1-deficient cells and mice also have restored growth, decreased chromosomal aberrations, increased RAD51 foci formation and at least partially restore HR relative to Brca1-deficient mice. Importantly, the loss of 53bp1 in Brca1-deficient mice renders them resistant to PARP inhibitor. Additionally, a subset of olaparib-resistant Brca1/P-glycoprotein-

deficient murine tumours had lost 53bp1 expression, while several others had heterogeneous expression (Jaspers, et al. 2013). Bouwman et al also found reduced 53BP1 expression in clinical *BRCA1/2*-associated and triple negative breast cancers (Bouwman et al. 2010). Lower 53BP1 expression was correlated with lower metastasis free survival, presumably due to reduced response to therapy. Together these data suggested that 53BP1 loss in a BRCA1-deficient background is a mechanism of PARP inhibitor resistance in mice and humans. The prevalence of 53BP1 loss in patients with BRCA1-associated and triple negative breast cancer remains to be determined.

5.3 Replication fork protection

Studies investigating the mechanisms that mediate replication fork stability in the absence of *BRCA1* or *BRCA2* have led to a number of additional mechanisms of drug resistance to be proposed. As discussed earlier, replication forks in *BRCA1/2* mutant cells are liable to degradation via MRE11 (Pathania et al. 2014; Schlacher et al. 2011; Schlacher et al. 2012). Chaudhuri et al recently found that that in *Brca2* mutant cells, loss of PTIP improved cell viability, protected HU-stalled replication forks from MRE11-mediated degradation and decreased genetic instability (Chaudhuri, et al. 2016). These effects were not caused by restoration of HR, but are best explained by PTIP's role in localising MRE11 to replication forks; in the absence of PTIP, replication fork degradation via MRE11 was reduced, which in turn led to a reduction in replication fork degradation. Chaudhuri et al also found that BRCA1/2-deficient cells with co-occurring PTIP defects also showed a reduced number of chromosomal abnormalities when exposed to either cisplatin or a PARP inhibitor, suggesting that these processes could influence BRCA1/2 mutant tumour cell response to therapy (Chaudhuri et al. 2016). Indeed, in a series of *Brca2*-deficient, PARP inhibitor resistant, mouse tumours, RAD51 foci formation was not restored but replication fork degradation after HU challenge was reduced, suggesting that HR

restoration was not the cause of drug resistance in this case, but that restoration of fork stability could be (Chaudhuri et al. 2016). Taken together, this data provide a case for assessing biomarkers of replication fork stability in clinical trials involving *BRCA1/2* mutant cancer patients.

5.4 PARP inhibitor resistance due to increased efflux

Increased efflux of PARP inhibitor from cancer cells also contributes to PARP inhibitor resistance in the *BRCA1/2* mutation context. Rottenberg et al showed that mammary tumours in *Brca1/p53* double-mutant mice that are initially very responsive to olaparib eventually become resistant to the drug (Rottenberg, et al. 2008). PARP inhibitor resistance in these tumours is mediated by increased expression of P-glycoprotein (Pgp) transporter genes *Abcb1a* and *Abcb1b* and can be reversed by inhibiting Pgp activity with tariquidar. Knock out of the Pgp *Mdr 1a/b* gene in a *Brca1* mutant background improved response of mammary tumours to PARP inhibitor, though they eventually became resistant due to other mechanisms (Jaspers et al. 2013). Additionally, multidrug resistance, including to olaparib, observed in a *Brca2*-mutated mouse model of mammary mesenchymal carcinosarcomas was, in part, due to increased Pgp expression (Jaspers, et al. 2015). Though increased efflux via Pgp transporter upregulation leads to PARP inhibitor resistance in *Brca1* and *Brca2* mutant mouse models of breast cancer, it has yet to be reported in the clinic.

6. BRCAness

In addition to patients with germline *BRCA1* or *BRCA2* gene mutations (*gBRCA*), it seems very likely that significant numbers of cancer patients without *gBRCA* mutations have tumours that resemble, at the molecular and histological level, *gBRCA* mutant tumours, a phenomenon termed BRCAness (reviewed recently in (Lord and Ashworth 2016)). In some cases, these shared molecular features might also drive the same defect in HR that could lead to sensitivity to BRCA synthetic

588 lethal treatments such as PARP inhibitors.

589 BRCAness might be driven by several different mechanisms. With the onset of large-
590 scale tumour sequencing, it is clear that in addition to germ-line *BRCA* gene
591 mutations, a significant proportion of non-familial cancers have somatic alterations in
592 *BRCA1*, *BRCA2* or the growing number of genes associated with HR. For example,
593 triple negative breast cancers, HGSOvCa, metastatic, castration-resistant prostate
594 cancer and pancreatic ductal adenocarcinomas exhibit somatic alterations in *BRCA1,*
595 *BRCA2* or BRCAness genes such as *ATM, ATR, BAP1, CDK12, CHK1, CHK2*, the
596 Fanconi anemia proteins (*FANCA, C, D2, E, F), PALB2*, *NBN, WRN*, the *RAD51*
597 homologs *RAD51B, C* and *D, MRE11A, BLM* and *BRIP1* (reviewed in (Lord and
598 Ashworth 2016)). Many of these genes have been shown in pre-clinical models to
599 cause PARP inhibitor sensitivity when dysfunctional (Bajrami, et al. 2014; Blazek, et
600 al. 2011; Joshi, et al. 2014; McCabe, et al. 2006), extending the causative link
601 between HR dysfunction and sensitivity to these drugs.

602 There is also growing evidence for BRCAness in tumours that have a particular
603 spectrum or pattern of mutations. One of the key observations made from the
604 genomic profiling of tumours is the classification of tumours according to the type of
605 mutations they possess, a *mutational scar*, rather than the specific genes that are
606 mutated. In some instances, these mutational scars reflect the natural history of a
607 tumour, and particularly the types of DNA damage and repair that have molded the
608 genome over successive cell cycles. For example, *BRCA1* and *BRCA2* mutant
609 tumours exhibit a mutational scar that appears to be caused by the elevated use of
610 NHEJ, a DNA repair process that predominates in the absence of HR. For example,
611 recent work from Nik-Zainal and colleagues, based on data from the whole-genome
612 sequences of 560 breast tumours, confirmed the presence of three distinct genomic
613 rearrangement signatures associated with loss of HR in tumours, each characterised
614 by tandem DNA duplications or deletions; one of these signatures appears to be

associated with loss of BRCA1 function, the second being associated with defective BRCA1 or BRCA2, with the etiology of the third signature remaining largely unknown (Nik-Zainal, et al. 2016). The discovery of these genomic signatures in part reflects observations made in genetically engineered mouse cell lines with either *Brca1* or *Brca2* mutations, where the use of non-conservative forms of DNA repair such as NHEJ results in an elevated frequency of DNA deletions flanked by short, tandem DNA repeats at the break points of the deletion (Moynahan et al. 2001b; Tutt, et al. 2001; Xia, et al. 2001). Similar mutational scars to those seen in *gBRCA* mutant tumours are also seen in non-*gBRCA* mutant tumours, and even in those without a detectable germline or somatic alteration in an HR gene, suggesting that similar DNA repair defects might be operating in these tumours. Importantly, there is now a growing body of evidence that suggests that the presence of such BRCAness mutational scars also correlates with clinical responses to HR targeting agents such as platinum salts and PARP inhibitors (Birkbak, et al. 2013), correlations which are driving the development of clinically applicable BRCAness mutational scar assays. Most of these assays use genome-wide DNA copy number profiling to estimate the extent of chromosomal rearrangements characteristic of an HR defect (Birkbak et al. 2013).

6. Extending the utility of the synthetic lethal paradigm

It seems reasonable to question whether synthetic lethality as a concept might have a wider applicability in the search for optimised treatments for breast cancer. The progress in the molecular profiling of breast tumours means that there is now a working list of driver gene defects in the disease that in principle could be targeted with a synthetic lethal approach. For example, many of the tumour suppressor gene defects that recurrently occur in breast cancer, such as *TP53, PTEN* and *RB1* might be amenable to synthetic lethal approaches and already a number of candidate

642 synthetic lethal targets for these genes have been identified (Edgar, et al. 2005;
643 Emerling, et al. 2013; Gordon and Du 2011; Mendes-Pereira, et al. 2012; Mereniuk,
644 et al. 2013; Morandell, et al. 2013; Origanti, et al. 2013; Reaper, et al. 2011). Many of
645 the efforts to identify synthetic lethal interactions that are relevant to breast cancer
646 have been driven by advances in functional genomic approaches such as RNA
647 interference screening and more recently CRISPR based screens (Gilbert, et al.
648 2014; Morgens, et al. 2016; Wang, et al. 2015). The synthetic lethal approach might
649 also be applied to target relatively common oncogene amplification events in breast
650 cancer such as *MYC* amplification, which is present in over 22% of all breast tumours
651 (Cerami, et al. 2012; Ciriello, et al. 2015; Gao, et al. 2013). *MYC* encodes a
652 transcription factor, which might be challenging to directly target with drug-like small
653 molecules, and so employing synthetic lethal strategies to targeting *MYC*
654 amplification seems a reasonable approach. Already synthetic lethal interactions
655 between *MYC* and the DR5 death receptor pathway (Wang, et al. 2004) or inhibition
656 of the splicesome in MYC-dependent breast tumours have been identified (Hsu, et al.
657 2015). This latter observation might be explained by an increased dependency in
658 MYC amplified tumours upon pre-mRNA processing (Hsu et al. 2015).
659
660 Whilst there are clearly opportunities to more widely exploit the synthetic lethal
661 concept in breast cancer, there are also clear challenges. For a synthetic lethal effect
662 to be clinically actionable and to have significant utility, there are certain qualities the
663 synthetic lethal relationship must exhibit, many of which are common to all ideal
664 therapeutic approaches, synthetic lethal or not. Firstly, the therapeutic window
665 between tumour and normal cell inhibition/toxicity achieved with the synthetic lethal
666 target must be profound. Secondly, ideal synthetic lethal effects must be highly
667 penetrant – i.e. the presence of the predictive biomarker (e.g. a mutation in a breast
668 cancer driver gene) must be highly predictive of sensitivity to inhibition of the
669 synthetic lethal target; if this is not the case then a novel synthetic lethal treatment

might only work in a minority of patients or a minority of tumour cell clones within an individual. Thirdly, ideal synthetic lethal interactions must be relatively resilient to additional molecular changes that might reverse the synthetic lethal effect; this is critical if clinical synthetic lethal effects are to be effective in breast tumours, whose inherent molecular heterogeneity and ability to evolve and survive in the face of negative selective pressure is well documented (Alizadeh, et al. 2015; Brooks, et al. 2015). Despite advances in the ability to identify synthetic lethal effects in breast tumour cells, somewhat less attention is often given to whether these effects also fulfill these ideal criteria.

One concept that might gain further scrutiny in the future is the idea of exploiting combinations of different synthetic lethal effects in the same tumour, each of which focuses on a different breast cancer driver gene or phenotype. For example, in triple negative breast cancers with germline or somatic *BRCA* gene mutations, *TP53* mutations also co-occur. It seems reasonable to suggest that a drug combination strategy that involves a PARP inhibitor (to synthetically lethal target the *BRCA* gene defect), used alongside a *TP53* synthetic lethal therapy, might be more effective than PARP inhibitor monotherapy which might be limited by the emergence of secondary mutant *BRCA1/2* alleles. This idea of targeting multiple co-occurring driver mutations in the same tumour might be most effective when mutations that occur early on in the disease process, and so are more likely present in the majority of subclones in a tumour, are selected.

7. Conclusions and future prospects

The cloning of *BRCA1* and *BRCA2* stimulated a large body of work, from many investigators, that ultimately resulted in the first clinically approved treatment for a genetically defined cancer syndrome. Whilst this work provides a very compelling

narrative that illustrates the impact pre-clinical and clinical research can have, several important questions still remain. Some of these pertain directly to the use of PARP inhibitors whilst others are also relevant to the treatment of cancer in general. For example, although olaparib has been approved for use as a maintenance therapy after platinum treatment in HGSOv cancer, a role for first line PARP inhibitor treatment in *BRCA1* or *BRCA2* mutant patients, or those with BRCAness, remains to be established. There is also very little understood about what might constitute the optimal drug combination strategies involving PARP inhibitors or how patients with PARP inhibitor resistance might best be treated. It seems reasonable to suggest that some of the answers to these questions will come from clinical studies but also will be informed by pre-clinical research and a continued focus on the molecular biology of the BRCA1 and BRCA2 genes. More generally, the wider clinical applicability of the synthetic lethal concept is still not established, although it is hoped the continued pre-clinical research activity in this area will ultimately lead to further clinical trials drug approvals that deliver more effective treatments of cancer patients.

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Figure and Table legends

Figure 1. Oncogene addiction and synthetic lethality. Oncogene and tumour suppressor gene mutations drive the oncogenic process. As well s driving the oncogenic process, alterations in oncogenes and tumour suppressor also impart upon tumour cells a distinct set of genetic dependencies not present in normal cells, known as oncogene addictions, non-oncogene addictions and synthetic lethal effects. **A**. Oncogene addiction is the situation where a tumour cell becomes totally dependent on the activity of a mutated gene (Gene A is shown as an example). An analogous scenario, known as non-oncogene addiction exists when tumour cells with an alteration in an oncogene A, become addicted to the activity of a non-oncogene, B. **B**. Synthetic lethality is a scenario where loss of either gene A or gene B function is tolerated but simultaneous loss of both genes is not. In normal cells inhibition of either A or B does not result in cell death. In tumour cells where gene B is rendered dysfunctional (for example by mutation) inhibition of gene A results in cell death.

Figure 2. DNA lesions causing replication fork collapse. A working model of the DNA damage response (DDR) to replication fork stalling agents is shown. Drug-like PARP inhibitors trap PARP on DNA. Likewise, Topoisomerase poisons trap

topoisomerase enzymes on DNA. Platinum salts cause DNA cross-links. Each of these events stalls the progression of replication forks in S phase. Stalled forks often collapse, forming DNA double strand DNA breaks. DSBs in this setting are often cytotoxic if not repaired. The normal DNA repair process, homologous recombination, is controlled by BRCA1 and BRCA2. BRCA1 function is required for the processing of DNA ends prior to repair, a process known as end resection. Once end resection is complete, BRCA2 localises the key DNA recombinase enzyme, RAD51, to DNA at the site of DNA damage. The binding of RAD51 to DNA allows damaged DNA to invade an intact DNA double helix with homologous DNA sequence to that at the site of DNA damage (often in the sister chromatid), which is used as a template upon which to synthesise new DNA as part of the DNA repair process. In the absence of functional BRCA1 and BRCA2, cells either fail to effectively repair DNA (which can lead to apoptosis), or to utilise orthogonal DNA repair processes such as Non Homologous End Joining, which increase the frequency of complex DNA rearrangements, events that ultimately impair the fitness of cells.

Figure 3: Mechanisms of PARP inhibitor resistance in BRCA1/2-associated cancers. Loss of PARP inhibitor resistance in BRCA1/2 mutated cancers can occur via (1) restoration of BRCA1/2 function and HR by secondary intragenic BRCA1/2 mutations, expression of hypomorphic BRCA1 alleles, stabilization of mutant BRCA proteins and demethylation of the *BRCA1* promoter, (2) restoration of HR as a result of relief from 53BP1 mediated block on end-resection (only in *BRCA1* mutant tumour cells), (3) protection of replication forks, from MRE11-mediated degradation, due to loss of PTIP, CHD4 or PARP1 expression and (4) increased efflux of PARP inhibitor from cancer cells as a result of increased P-glycoprotein expression.

Declaration of interest statement

CJL is a named inventor on patents describing the use of PARP inhibitors and stands to gain from their use as part of the ICR Rewards to Inventors Scheme.

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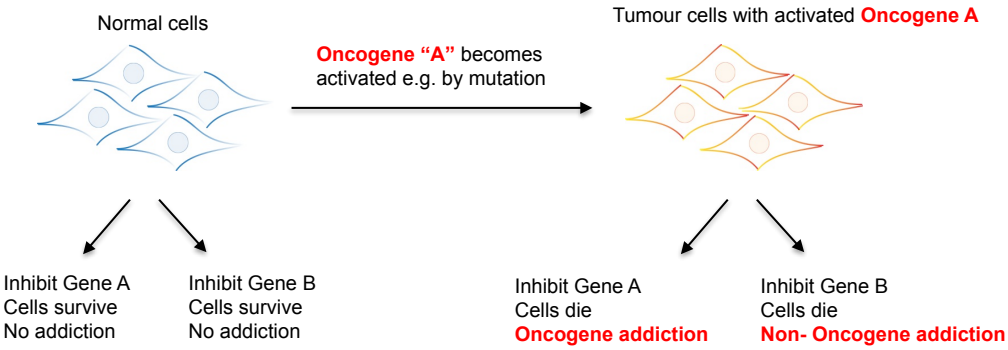
Figure 1.

A.



B.

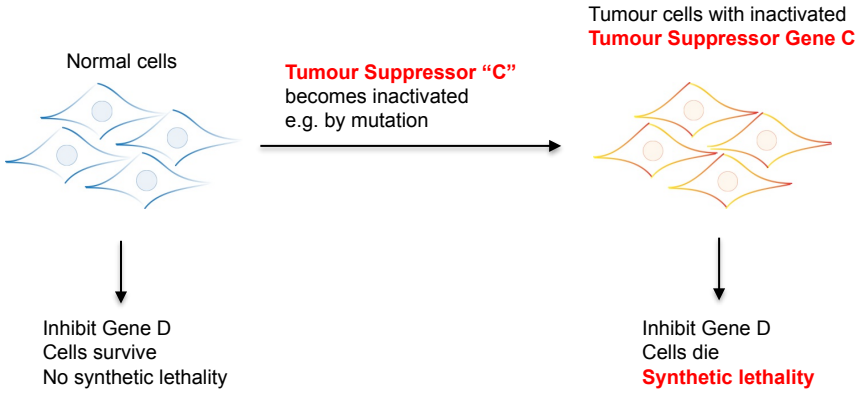


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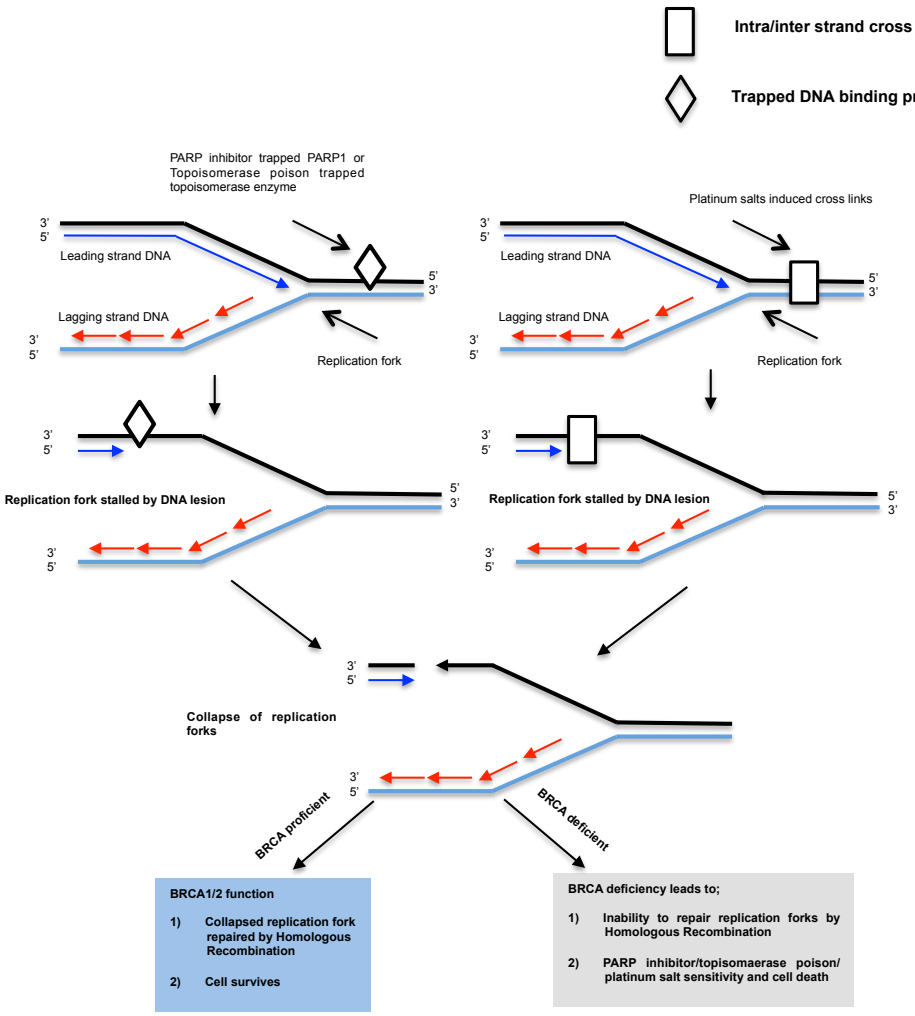
Figure 2



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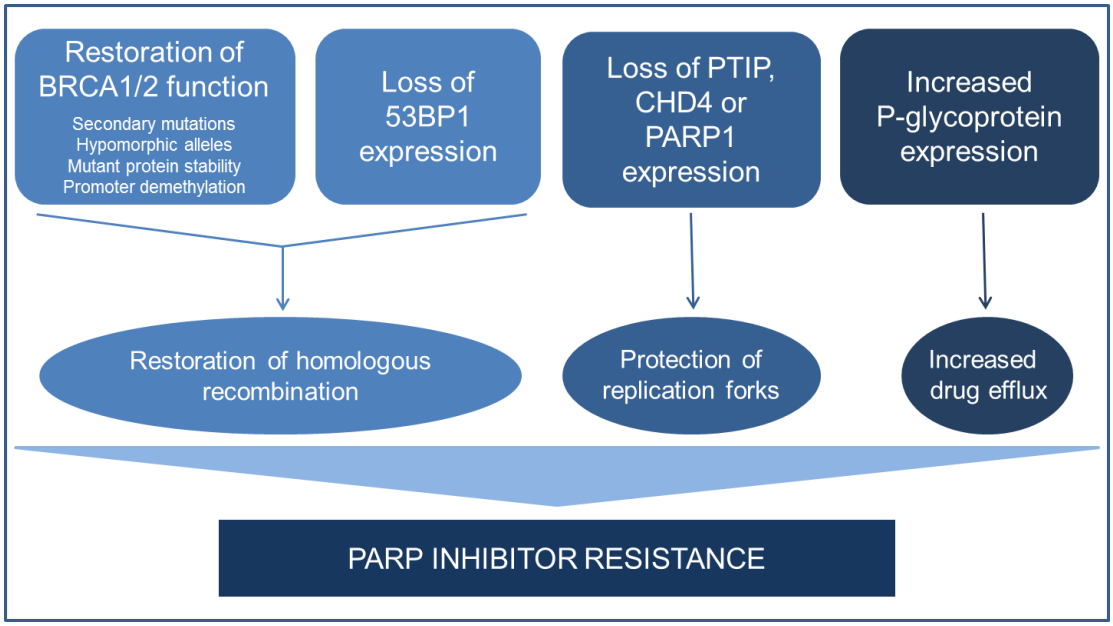
Restoration of BRCA1/2 function
Secondary mutations
Hypomorphic alleles
Mutant protein stability
Promoter demethylation
Loss of 53BP1 expression
Loss of PTIP, CHD4 or PARP1 expression
Increased P-glycoprotein expression
Restoration of homologous recombination
Protection of replication forks
Increased drug efflux
PARP INHIBITOR RESISTANCE

1382